National Toxicology Program
Toxicity Report Series
Number 21

NTP Technical Report
on Toxicity Studies of

2-Hydroxy-4-methoxybenzophenone

(CAS Number: 131-57-7)

Adminstered Topically and in Dosed Feed
to F344/N Rats and B6C3F$_1$ Mice

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The NTP Report on the Toxicity Studies of 2-Hydroxy-4-methoxybenzophenone is based primarily on 2- and 13-week studies that began in June, 1985, and concluded in July, 1988, at EG&G Mason Research Institute, Worcester, MA.

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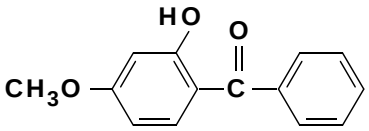
2-Hydroxy-4-methoxybenzophenone



CAS Number: 131-57-7

Molecular Weight: 228.26

Synonyms: Oxybenzone; 4-Methoxy-2-hydroxy-benzophenone; Cyasorb UV; Uvinul M 40; (2-hydroxy-4-methoxyphenyl)phenyl-methanone; NSC-7778; Spectra-sorb UV; Syntase 62; UF 3; USAF CY-9; NCI-C60957

ABSTRACT

2-Hydroxy-4-methoxybenzophenone (HMB) occurs naturally in flower pigments and is synthesized for use in sunscreens, as a UV stabilizer in various cosmetic products, and in plastic surface coatings and polymers. Toxicity studies of HMB were performed in F344/N rats and B6C3F$_1$ mice, by administering HMB in feed and by topical application, in studies of 2 weeks' (5 animals/sex, dose and species) and 13 weeks' (10 animals/sex, dose and species) duration. Assessments included hematology, clinical chemistry, urinalysis, reproductive toxicity, and histopathologic evaluations.

In both 2- and 13-week dosed feed studies, rats received diets containing 0, 3125, 6250, 12500, 25000, or 50000 ppm HMB. One high-dose female rat died during the 2-week study. Body weight gains of high-dose male and female rats were reduced in the 13-week study. Liver and kidney weights were increased in dosed rats in both studies. In the 2-week studies, enlarged livers were associated with a marked hepatocyte cytoplasmic vacuolization in rats receiving diets containing concentrations of 6250 ppm HMB or higher; renal lesions, consisting of dilated tubules and regeneration of tubular epithelial cells, were found primarily in high-dose rats. In the 13-week studies, kidney lesions progressed to include papillary degeneration, or necrosis, and inflammation, while the liver lesion appeared to regress; liver enzymes in serum remained elevated. Rats receiving a diet with 50000 ppm HMB showed markedly lower epididymal sperm density and an increase in the length of the estrous cycle at the end of the 13-week studies.

In 2-week dermal studies, rats received topical applications of 1.25 to 20 mg of HMB in an acetone or lotion vehicle. The only effects noted were small and variable increases in liver and kidney weights, reaching statistical significance primarily in the higher dose groups. In 13-

week studies, rats received topical doses from 12.5 to 200 mg/kg HMB in acetone. Kidney weights were elevated in dosed groups of female rats. No other findings were attributed to HMB treatment.

In 2- and 13-week dosed feed studies, mice received feed containing 0, 3125, 6250, 12500, 25000, or 50000 ppm HMB. A dose-related increase in liver weight associated with hepatocyte cytoplasmic vacuolization was the only finding in mice in the 2-week studies. Decreased body weight gains were dose-related in mice in the 13-week studies; mild increases in liver weights were seen in dosed mice of both sexes. Kidney weights were increased variably in dosed females. Microscopic lesions were noted only in the kidneys of males receiving 50000 ppm HMB; these included eosinophilic protein casts in dilated renal tubules and a mild inflammation associated with the dilated tubules. Mice in the highest dose group exhibited a decrease in epididymal sperm density and an increase in length of the estrous cycle.

In 2-week dermal studies, mice received topical applications from 0.5 to 8 mg HMB in an acetone or lotion vehicle. The only effects noted were minimal, variable increases in liver and kidney weights, primarily in the higher dose groups. In 13-week studies, mice received topical doses of 22.75 to 364 mg/kg in acetone. Kidney weights were increased variably in dosed male mice. Epididymal sperm density was decreased at all 3 dose levels evaluated (22.75, 91, and 200 mg/kg).

The genetic toxicity of HMB also was evaluated in mutagenicity studies with *Salmonella typhimurium*, in cytogenetic studies with Chinese hamster ovary (CHO) cells, and by evaluation of micronucleated erythrocytes in peripheral blood smears from mice in the 13-week studies. HMB was weakly mutagenic in *Salmonella* with metabolic activation, and induced sister-chromatid exchanges and chromosomal aberrations in CHO cells in the presence of a metabolic activation system. There was no increase in the frequency of micronucleated erythrocytes in the blood of mice receiving HMB.

In summary, HMB produced generally similar effects following topical and oral administration to rats and mice. Consistent findings included decreases in epididymal sperm density, lengthened estrous cycle, and increased liver and kidney weights. Mice in the dosed feed studies exhibited microscopic changes in the kidneys, comprising tubular dilatation with eosinophilic protein casts. Dilatation, tubular regeneration, papillary degeneration, and inflammation were noted in the kidneys of rats; and liver lesions consisting of an apparently reversible hepatocyte cytoplasmic vacuolization occurred in both rats and mice. A no-observed-adverse-effect level (NOAEL) for microscopic lesions was 6250 ppm HMB in the diet for rats and mice. A NOAEL was not reached for decreased epididymal sperm density in the 13-week dermal study in mice (<23 mg/kg/day).

PEER REVIEW

Peer Review Panel

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies on 2-hydroxy-4-methoxybenzophenone on November 21, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report fully and clearly presents the experimental results and conclusions.

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Summary of Peer Review Comments

Dr. J.E. French, NIEHS, introduced the short-term toxicity studies of 2-hydroxy-4-methoxy-benzophenone (HMB) by reviewing the natural occurrences and uses of HMB, experimental design, and results. Review of unpublished proprietary information as well as FDA files led to the decision to use a sunscreen lotion base as a dose vehicle and to use both oral and dermal routes of exposure. Additionally, liver, kidney, and male and female reproductive organs were identified as target organs; only the kidney had been indicated in published literature.

Dr. Carlson, a principal reviewer, remarked that this report dealt with 8 studies and that a good job was done of handling a lot of data. He suggested that use of the term, "topical application," was more common and would be more correct than "dermal application." Dr. French commented that the NTP historically has used "dermal" as specific to skin, while "topical" could apply to other sites, e.g., the eye. Dr. Carlson noted that liver function was not determined as stated in the abstract, and that the enzyme changes were measures of damage.

Dr. Goodman, a second principal reviewer, commented that the report was well-written and the results clearly presented. He asked that a clearer rationale be given as to why the study was performed in view of the mention in the report that both an FDA panel and the Cosmetic Ingredient Panel concluded that HMB was safe with regard to its current uses. Dr. French responded that HMB was selected from a review of the ether chemical class study and was nominated primarily on the basis of human exposure and as a representative benzophenone derivative used as a UV screen and UV stabilizer. Dr. Goodman said it would be useful to indicate how the doses that produced toxicity compared with the dose one might anticipate from the "safe" human use of HMB. Dr. French remarked that this would be difficult to do; however, at least in the 2-week dermal studies, a lotion vehicle was used with HMB concentrations which represented the maximum amount to be applied in a sunscreen lotion to human skin.

Dr. Carlson commented that there appeared to be too much emphasis placed on the lack of a NOAEL for decreased epididymal sperm density in the 13-week dermal study in mice. Dr. Richard Davis, American Cyanamid, suggested adding other measures of male reproductive function, such as spermatid counts, in addition to sperm density counts. He said that this would improve the consistency in reporting, noting that for the 4 studies being reviewed there was a three-fold range for control groups alone in sperm density. Dr. B. Schwetz, NIEHS, reported that, since the time the HMB studies were conducted, spermatid head counts were being collected as a reflection of the activity of the spermatogenesis process. Dr. French responded that comparisons between reproductive endpoints collected in different laboratories are made, but that primary emphasis is placed on the concurrent control for interpretation and conclusion. [Subsequent studies of the effect of topically applied HMB on sperm production and characteristics in B6C3F_1 mice, sponsored by the Cosmetic Toiletry and Fragrance Association, failed to show statistically significant decreases in epididymal sperm density or other effects on the reproductive system (Daston, G.P. Gettings, S.D., Carlton, B.D. *et al.*,

(1992) Assessment of the reproductive toxic potential of dermally applied 2-hydroxy-4-methoxybenzophenone to male B6C3F_1 mice, Fundam. Appl. Toxicol., in press].

Seeing no objections, Dr. Klaassen accepted the report with the suggested editorial and other changes on behalf of the panel.